MCMR-UIZ-L (70-1n)

12 February 1996

MEMORANDUM THRU Chief, Virology Division

FOR Dr. George Ludwig, Virology Division

SUBJECT: Protocol Approval V96-01 -

1. Your protocol (copy enclosed) entitled "Vaccine Immunogenicity and Efficacy Testing in Mice" has been approved and assigned Protocol Number V96-01. Please use this protocol number when ordering animals, submitting samples to the clinical laboratory, submitting samples for pathological evaluation, etc.

2. Please notify Mrs. Loberta Staley when this protocol has been completed so the animal account can be closed and the "Report of Protocol Completion/Termination" can be completed and submitted. Also, please advise the LACUC Chairman if there are any changes in personnel, the experimental design, the animal procedures/manipulations, the animal pain status, or a significant change in the number of animals authorized so that the protocol record may be properly annotated.

Encl

GERALD P. JAAX
COL, VC
Chairman, LACUC
Veterinary Medicine Division

EXHIBIT P

USAMRIID RESEARCH PROTOCOL

Proposal No. 96-13 Protocol No V96-01

Date Approved: 12 Feb 96

APC: MIIB

Title: Vaccine immunogenicity and efficacy testing in mice

Principal Investigator(s)/Division/Phone #:
George V. Ludwig, Ph.D., Virology Division, 4941 2/6

Scientific/Division Review:

Recommend Approval: date 1-25-96
(Division Chief signature)

Attending/Consulting Veterinarian:

date 1-25-96
(signature)

Statistical Review:

Jane Nelson date 24 Jan 96
(Biostatistican signature)

LACUC:

Recommend Approval:
~~Recommend Disapproval~~:

date 5 Feb 96
(subcommittee chairman)

Recommend Approval:
~~Disapproved~~:

date 5 Feb 96
(committee chairman)

Commander:

Approved:
Disapproved:

date 10 Feb 96
(signature)

PROTOCOL NUMBER: V96-01

PROTOCOL TITLE: Vaccine immunogenicity and efficacy testing in mice

PRINCIPAL INVESTIGATOR(S)/DIVISION(S):
George V. Ludwig, Ph.D., Virology Division

CO-INVESTIGATOR(S)/DIVISION(S):
Jonathan F. Smith, Ph.D., Virology Division
Peter Pushko, Ph.D., Virology Division
LTC William D. Pratt, DVM, Ph.D., Virology Division

I. NON-TECHNICAL SYNOPSIS: Development of vaccines capable of protecting troops against biowarfare threats and infectious diseases is an important component of the USAMRIID mission. We currently have many vaccines that are in various levels of development. Such vaccines include a new generation of alphavirus vaccine derived from a genetically engineered infectious cDNA clone, the alphavirus replicon system, and killed, microencapsulated preparations. Basic testing of these vaccine candidates require immunogenicity and efficacy testing in appropriate animal models. This protocol details testing of new vaccines currently under development in the Virology Division. We are currently ready to test vaccines that should induce immune responses against proteins from Lassa fever, influenza, and Rift Valley fever viruses. As many as 14 additional vaccines will be available within the next year. To complete efficacy studies, varying concentrations of vaccine preparation will be inoculated into groups of appropriate animals. After 28 days the animals will receive a booster immunization followed in 28 more days by challenge with virulent homologous virus. Blood samples will be obtained from animals prior to the first immunization, before boosting, before challenge, and 28 days post-challenge if necessary (for survivors). Survivalship data and serologic analysis will then be used to direct further vaccine development efforts.

II. BACKGROUND:

A. Background: Development of vaccines capable of protecting troops from a variety of infectious and non-infectious disease causing agents is one of USAMRIID's most important missions. Such vaccines have been developed using a variety of approaches including classical procedures for inactivation and development of attenuation. More recent advances in vaccinology have led to the development of new procedures for vaccine development. These include a variety of subunit approaches, live virus infectious clones, naked DNA, and replicon systems. All of these approaches have their own advantages and disadvantages and the decision on which system to use depends on the nature of the disease causing agent, the mode of transmission and a myriad of additional factors. Regardless of the type of vaccine being developed, all vaccines must be tested at an early stage to determine if they will be immunogenic and effective in an appropriate animal model. Many of the infectious and non-infectious agents being researched at USAMRIID produce lethal infections in mice, although the strain of mouse used and the route of challenge will vary from agent to agent. Some, like Rift Valley fever virus produce lethal infections in most strains of mice following a parenteral (subcutaneous or intraperitoneal) inoculation. Others, like vaccinia virus must be administered intracranially in order to produce a lethal disease. Still others, like influenza only produce a lethal infection when inoculated intranasally in outbred mouse strains.

Some agents for which vaccines are being developed, do not produce lethal infections in any animal other than humans. For these agents, potential vaccines can only be tested for immunogenicity. Immunological reagents for the mouse are readily available making monitoring of immune responses to vaccination easy to measure. This protocol outlines procedures for immunogenicity and efficacy testing of vaccine candidates currently under development in the Virology Division, USAMRIID in inbred and outbred strains of mice.

B. **Literature Search**:

1. **Literature Source(s) Searched**:

Medline
DTIC
FEDRIP

2. **Date and Number of Search**:

Medline - 12/12/95 (No search number given)
DTIC - 12/14/95 (Search number 3H025E)
FEDRIP - 12/14/95 (No search number given)

3. **Key Words of Search**:
Vaccine/VEE/WEE/EEE/Dengue/Rift Valley/Ebola/Mouse

4. **Results of Search**: No studies utilizing similar vaccine approaches are currently being undertaken. The results show that the proposed studies do not inappropriately replicate any past or ongoing animal research efforts.

III. **OBJECTIVE\HYPOTHESIS**: The objective of these studies is to: 1) measure the magnitude of humoral responses in mice to vaccine candidates currently under development in the Virology Division, USAMRIID. 2) Test the ability of these vaccines to protect mice from a lethal challenge when such a lethal challenge is appropriate.

IV. **MILITARY RELEVANCE**: Infectious disease is traditionally a more important impediment to troop readiness than any other cause of illness or injury. In addition, many infectious and non-infectious disease causing agents are classical biological warfare agents. As a result, the military has expended considerable effort in the preparation of effective vaccines which include both live-attenuated and killed preparations. However, many current products have serious shortcomings. The killed vaccines may not protect against an aerosol challenge and require multiple immunizations. Live-attenuated vaccines often have relatively high rates of adverse effects. For these reasons improved vaccines are highly desirable. The development of vaccines against infectious and non-infectious disease causing agents is, therefore, a major mission of the Medical Research and Material Command. Testing of these vaccines remains an important component of the development process and, therefore, is highly relevant to military planners and tacticians.

V. MATERIALS AND METHODS:

A. Experimental Design and General Procedures:

Testing of fifteen vaccine candidates is currently planned. This includes twelve vaccines prepared in the alphavirus replicon system: vaccinia, Lassa fever, Ebola, Marburg, influenza, Rift Valley fever (RVFV), Crimean-Congo hemorrhagic fever (CCHF), sandfly fever, and the four dengue serotype viruses. Additionally three microencapsulated vaccines: Eastern (EEE), western (WEE) and Venezuelan equine encephalitis (VEE) viruses will be tested. Each vaccine will be administered in 6 doses (five vaccine dilution groups and one unvaccinated control group) to 10 mice each. For comparison, 10 additional mice will be inoculated with any current vaccine that may be available (influenza, RVFV, EEE, WEE, and VEE). Therefore, this protocol will require 950 mice.

Vaccine	Vaccination Groups	Negative Control	Positive Control	Total
Vaccinia	50	10		60
Lassa fever	50	10		60
Ebola	50	10		60
Marburg	50	10		60
Influenza	50	10	10	70
RVFV	50	10	10	70
CCHF	50	10		60
Sandfly fever	50	10		60
Dengue 1	50	10		60
Dengue 2	50	10		60
Dengue 3	50	10		60
Dengue 4	50	10		60
EEE	50	10	10	70
WEE	50	10	10	70
VEE	50	10	10	70
				950

1. Vaccination: Vaccines will be delivered in one of two forms. The first will be immunogen in sterile saline solution, while the second will be immunogen in polylactide-coglycolide microcapsules. For the former, the effect of dose on immune responses will be tested by diluting vaccine in sterile Hank's balanced salt solution. For the latter, specific concentrations of immunogen will be calculated from protein assay data obtained during microcapsule preparation. In either case 200 ul of 5 different vaccine concentrations will be inoculated subcutaneously into groups of 10 mice using a 1 cc syringe with 26g x 5/8" needle. For each experiment, another group of ten control mice will receive 200 ul of diluent alone. For the purpose of comparison, another group of 10 mice will receive currently available vaccine preparation (IND or licensed product if available) administered as described in product insert. When appropriate, booster immunizations will be administered 28 days post primary vaccination using the same procedure.

2. Serology: Mice will be bled from the retro-orbital sinus 2 days pre-immunization and as often as every 2 weeks up to the day of challenge. Any mice surviving challenge will be bled when all possibility of death resulting from challenge has passed, typically 21 days post-challenge. Minimally, mice will be bled 2 days prior to immunization, 2 days prior to boosting, and 2 days prior to challenge. Mouse serum will be separated from the clot and frozen at -20C until appropriate serological testing can be completed.

3. Challenge: Many of the agents being tested in this vaccination protocol do not produce lethal infections in mice. Therefore, challenge following vaccination will be completed only with animals immunized against vaccinia, RVFV, sandfly fever virus, VEE, WEE, and EEE.

a. IP (VEE, EEE, and WEE viruses): Mice to be challenged via the intraperitoneal route will be inoculated with 200 ul of agent being tested using a 1 cc syringe with 26g x 5/8" needle. Agents will be diluted in sterile Hank's balanced salt solution. Challenge doses to be used in these experiments will be obtained from results of previous experiments, but will always be sufficient to kill all control animals (typically 5 x 10^4 PFU/mouse). Mice will be monitored daily for 21 days for signs of illness or death.

b. SQ (RVFV): Same procedure as for IP only delivered subcutaneously.

c. IC (vaccinia and sandfly fever virus): Mice will be inoculated with 25 ul of agent intracranially using a 200 ul Hamilton syringe fitted with a 27g x 5/8" needle. Challenge doses to be used in these experiments will be obtained from results of previous experiments, but will always be sufficient to kill all control animals (typically 5 x 10^4 PFU/mouse). Mice will be monitored daily for 21 days for signs of disease or death. Detailed procedures for IC inoculation are given in section VI.C.3.a.

d. IN (influenza virus): For intranasal challenge 20 ul of agent (10 ul per nostril) will be administered per mouse using a 250 ul Hamilton syringe fitted with blunt ended canula. Mice will be monitored daily for 21 days for signs of disease or death. To accurately assess illness, mice will be weighed daily for 14 days to look for signs of wasting. Detailed procedures for IN challenge are given in section VI.C.4.

B. Laboratory Animals Required and Justification:

BALB/c mice (Lassa, Marburg, Ebola, RVFV, Dengue 1-4, VEE) C3H mice (vaccinia), C57/bl/6 mice (EEE and WEE), Swiss-Webster outbred mice (influenza)

1. **Non-animal Alternatives Considered:** No non-animal alternatives exist for this type of experiment. The only mechanism to test for the ability of a vaccine candidate to protect animals from an otherwise lethal challenge is to test the vaccine in a host in which virulence can be measured. Unfortunately, no accurate in vitro correlates to virulence of these agents have been identified.

2. **Animal Model and Species Justification:** While a number of animal models are appropriate for the type of experiments outlined in this protocol, the mouse is the lowest on the evolutionary scale. Some of the disease causing agents to be tested behave differently in different mouse strains. The choice of a particular strain will therefore depend on the susceptibility of the strain for the agent being tested. In some cases, outbred mice are the most appropriate model.

3. **Laboratory Animals:**

a. **Genus & Species:**

Mus musculus,

b. **Strain/Stock:** BALB/c mice, C3H mice, C57/bl/6 mice, Swiss-Webster outbred mice

c. **Source/Vendor:** Lowest cost, USAMRIID/LACUC-approved source

d. **Age:** 6-8 weeks

e. **Weight:** N/A

f. **Sex:** Female

g. **Special Considerations:** N/A

h. **Other:**

4. **Total Number of Animals Required:**

a.	mice	950
	1. Balb/c	475
	2. C57/bl/6	158
	3. C3H	158
	4. Swiss/Web.	159

5. **Refinement, Reduction, Replacement:** Utilization of the mouse as the animal model for this experiment was selected because of its extensive use in other vaccine development experiments and the fact that it is the most primitive animal model in terms of ranking on an evolutionary scale. Non-human primates, which would be another suitable model, are less desirable because of status on the evolutionary scale, their initial cost, the cost of handling, and space requirements. The number of animals per group has been minimized, but is large enough to ensure statistical validity of results. No expressed product vaccine will be used in mice until it has been shown to produce appropriate antigen in vitro.

a. **Refinement:** Moribund animals will be euthanized to prevent unnecessary pain and distress which is a refinement.

b. **Reduction:** The number of animals per group has been minimized, but is large enough to ensure statistical validity of results.

c. **Replacement:**

C. **Technical Methods:**

1. **Pain:**

a. **Pain category:** (USDA Form 18-13)

(1) No Pain 760 **(#)** (Column C)

(2) Alleviated Pain 120 **(#)** (Column D)

All animals used in this study will be bled from the retro-orbital sinus at least 3 times. The pain with involved with this procedure will be alleviated by administration of Metofane by inhalation prior to the bleeding procedure. Some animals will be inoculated intracranially. These animals will be anesthetized using the sodium pentobarbital/ethanol procedure described below.

(3) **Unalleviated Pain or Distress** 70 (#)
(Column E)

Animals vaccinated with preparations for which good challenge models exist will be challenged as described. Some low dose vaccinated animals and all control animals will succumb to signs of disease. While these animals will be euthanized when signs of discomfort or distress become apparent (see Appendix 1), it is inevitable that some degree of pain will be realized. Administration of prototype vaccine preparations alone have not been shown to cause illness or death in mice. No pain, therefore, should be associated with vaccination.

b. **Pain Alleviation:**

(1) **Anesthesia/Analgesia/Tranquilization:**

Bleeding and IN inoculation - All animals involved with this protocol will be bled from the retro-orbital sinus. To avoid discomfort during this procedure and during IN inoculation, mice will be placed into a closed 500 ml jar containing metofane-soaked 4 x 4 gauze pads. Animals will be separated from direct contact with anesthesia by a physical barrior. Mice will remain in the chamber until fully anesthetized (approximately 1 min) at which time they will be removed for bleeding. Mice will be monitored by the investigators until fully recovered.

IC inoculation - Animals to be inoculated intracerebrally will be anesthetized with 71 mg/kg of pentobarbital (e.g., Nembutal) containing 9.2% ethanol intraperitoneally using a 26 g x 5/8" needle.

(3) **Paralytics:** None

c. **Alternatives to Painful Procedures:**

(1) **Source(s) Searched:** MEDLINE

(2) **Date of Search:** 12/12/95

(3) **Key Words of Search:** pain, surgery, challenge, vaccination,

(4) **Results of Search:** No appropriate alternative to the procedures described was identified following review of the results of the stated search.

d. **Painful Procedure Justification**: While serological data alone may provide good information on vaccine immunogenicity, these data will not adequately address the question of protection from disease or death. In addition, no vitro correlates to protection will accurately predict animal survival following challenge.

Although, by necessity, there are animals included in the unalleviated pain and distress category of this protocol, there will be a conscious effort by the P.I. and animal care staff to provide as much additional consideration for the comfort and well-being of the animals as is consistent with the scientific integrity of the study.

2. **Prolonged Restraint**: None

3. **Surgery**:

a. **Procedure**: Intracerebral inoculation. Mice will be anaesthetized IP using 10 ul per gram of body weight of the following solution:

1.3 ml Sodium Pentobarbital (65 mg/ml)
1.1 ml 100% Ethanol
9.5 ml Sterile Saline

Following induction, the top of the head will be disinfected with iodine based surgical disinfectant. A small (1-2 mm) incision will be made to the skull between the ears. Using a Dremel® Tool with 25 g drill bit, a hole will be bored through the skull. By adjusting the amount of bit protruding from the tool collet, the depth of the hole can be finely adjusted to prevent penetration of the dura. Drill bit and tool collet will be flame disinfected prior to each use. A 27 g x 5/8" needle attached to a 250 ul Hamilton syringe containing the inoculum will be inserted approximately 10 mm into the mouse cerebrum and 0.025 ml of inoculum will be injected. Following inoculation, the skin incision will be closed with n-butyl cyanoacrylate (Vetbond®). This procedure has been used with great success under protocol V95-03

b. **Pre- and Postoperative Provisions**: No preoperative care is required. Following intracerebral inoculation mice will be kept warm under an indirect heat lamp or warming pad until recovery from anesthesia is complete. The animal's eyes will be kept moist using an appropriate ocular lubricant. Mouse husbandry will be completed by Veterinary Medicine Division technicians. Animals will be monitored daily by the PI or Co-I until the end of the study.

c. **Location**: Surgery will be done in a laminar flow hood within BSL-3 Suite V-3.

d. **Surgeon/Qualifications**: Surgery will be performed by Dr. George Ludwig. Dr. Ludwig has more 14 years experience working with laboratory rodents. Additionally, Dr. Ludwig has completed a non-credit course on laboratory animal surgery at the University of Wisconsin.

e. **Multiple Survival Surgery Procedures**: None

(1) **Procedures**:

(2) **Scientific Justification**:

4. **Animal Manipulations**:

a. **Injections**:

IP Inoculation - For this procedure mice will be restrained manually. Using a 1 cc syringe with 26g x 5/8" needle, 200 ul of appropriate inoculum will be delivered through the abdominal wall into the peritoneal cavity.

SQ Inoculation - For this procedure mice will be restrained manually. Using a 1 cc syringe with 26g x 5/8" needle, 200 ul of appropriate inoculum will be delivered under the loose skin behind the neck.

IC Inoculation - described under surgical procedures.

IN Inoculation - Following metofane anesthesia (described above) mice will be held head-up in one hand. Using a 250 ul Hamilton syringe fitted with a small blunt ended canula, 10 ul of preparation will be administered in small droplets to each nostril. Air movement during inhalation will provide the mechanism of internalization.

b. **Biosamples**:

Blood samples - Following metofane anesthesia (described above) mice will be bled from the retro-orbital sinus using a 9 in Pasteur pipet. Approximately 250 ul of blood will be collected at each bleed.

c. **Animal Identification**: All animals will be identified by standard ear punch markings completed while animals are under Metofane anesthesia.

d. **Behavioral Studies**: None

e. **Other procedures**: None

5. **Adjuvants**: None

6. **Study Endpoint**: For animals tested for immune response to vaccination only, the study will end 28 days following final booster vaccination and final bleed. These animals will be euthanized according to procedures outlined below. Studies in which animals receive a potentially lethal challenge will end either at the first signs of terminal illness or major distress (see appendix 1) or 21 days following challenge. These animals will also be euthanized according to procedures outlined below. Every reasonable attempt to prevent animals from entering terminal stages of disease will be made to reduce potential pain and distress to the animal.

7. **Euthanasia**: Animals will be humanely euthanized upon study endpoint or when signs of discomfort or distress become apparent (see Appendix 1). The method of euthanasia will be exposure to 100% CO_2 for at least 4 min followed by cervical disslocation.

D. **Veterinary Care**:

1. **Husbandry Considerations**: All animals will be housed and cared for according to the NIH "Guide," or the Veterinary Medicine Division SOP. Animals will be observed daily for general health, humane treatment, and general conditions. Animals will be euthanized at the first sign of terminal illness or major distress (see appendix 1).

a. **Study Room**: Room V307, Building 1425.

b. **Special Husbandry Provisions**: Micro-isolators.

2. **Attending Veterinary Care**: Animals will be observed daily for general health, humane treatment, and general conditions. Animals will be euthanized at the first sign of terminal illness or major distress (see appendix 1).

3. **Enrichment Strategy**: N/A.

a. **Dogs**:

b. **Nonhuman Primates**:

E. **Data Analysis**: Data will be analyzed using probit analysis, survivalship analysis, and/or Fisher's exact tests.

F. **Record Keeping**: Records of animal manipulations will be maintained in a USAMRIID laboratory notebook and computer database created by the PI.

G. **Investigator & Technician Qualifications/Training:** All procedures described in this protocol will be conducted by either the principal investigator or the co-investigators.

Dr. George V. Ludwig is Chief, Department of Cell Culture and Hybridoma Production at USAMRIID. Dr. Ludwig is a graduate of the University of Wisconsin-Madison, where he received a Ph.D. in Veterinary Science and Entomology. During 14 years of relevant experience, Dr. Ludwig has worked extensively with the species used in this protocol, and has mastered all of the manipulations described.

Dr. Smith possess a Ph.D. in Microbiology and has performed animal manipulations, of the type described, many times during his 20 years of experience with the species used in this protocol.

Dr. Pushko possess a Ph.D. degree in molecular biology from University of Partu, Estonia. He has been fully trained to complete IP and SQ inoculations and bleeding procedures and has performed these procedures under the supervision of the PI for the last 2 years.

LTC William D. Pratt, DVM, Ph.D., is a veterinarian with 6 years of clinical experience and 7 years of research experience. This experience includes surgery, anesthesia, injections, bleeding, and euthanasia of avian, nonhuman primate and rodent species. He was fully trained to perform the animal manipulations specified below by VMD personnel.

H. **Time Lines:**

1. **Estimated Start Date:** February 1996

2. **Estimated Completion Date:** January 1999

VI. Biohazard/Safety: Certain vaccines (eg. replicon based vaccine delivery systems) have currently not been downgraded to BL-2 or less. Access requirements to V-3 will adequately protect animal caretakers and other investigators from any potential exposure to vaccines or challenge stocks used in this experiment.

VII. Extramural Collaboration: None

VIII. **COORDINATION**: Name, signature, and date for the appropriate person or office are required. Signature in these blocks are indicative of the supporting entities capability to provide the specific services or support detailed in the protocol, and should not be considered to be an approval of the project. (No response to the title paragraph of this section.)

A. Veterinary Support (Veterinary Medicine Division)

B. Aerobiology & Product Evaluation Support (Toxinology Division) N/A

C. Clinical Lab Support (Diagnostic Systems Division)

N/A

D. Pathology Division Support N/A

E. BDRP/ID STO Coordinator

Science & Technology Objective (STO) Title

F. Other Support

IX. **ASSURANCES**: The law specifically requires several written assurances from the P.I. It states that "research facilities will be held responsible if it is subsequently determined that an experiment is unnecessarily duplicative, and that a good faith review of available sources would have indicated as much." (No response under the title paragraph of this section.)

As the Principal Investigator on this protocol, I acknowledge my responsibilities and provide assurances for the following:

A. **Animal Use**: The animals authorized for use in this protocol will be used only in the activities and in the manner described herein, unless a deviation is specifically approved by the LACUC.

B. **Duplication of Effort**: I have made a reasonable, good faith effort to ensure that this protocol is not an unnecessary duplication of previous experiments.

C. **Statistical Assurance**: I assure that I have consulted with a qualified statistician to evaluate the statistical design or strategy of this proposal, and that the "minimum number of animals needed for scientific validity are used."

D. Biohazard\Safety: I have taken into consideration, and I have made the proper coordinations regarding all applicable rules and regulations regarding radiation protection, biosafety, recombinant issues, etc., in the preparation of this protocol.

E. Training: I verify that the personnel performing the animal procedures/manipulations described in this protocol are technically competent and have been properly trained to ensure that no unnecessary pain or distress will be caused to the animals as a result of the procedures/manipulations.

F. Responsibility: I acknowledge the inherent moral and administrative obligations associated with the performance of this animal use protocol, and I assure that all individuals associated with this project will demonstrate a concern for the health, comfort, welfare, and well-being of the research animals. Additionally, I pledge to conduct this study in the spirit of the fourth "R" which the DoD has embraced, namely, "Responsibility" for implementing animal use alternatives where feasible, and conducting humane and lawful research.

(Principal Investigator Signature)

G. Painful Procedures: A signature for this assurance is required by the P.I. only if the research being conducted will cause more than slight or momentary pain or distress (see page 5.V.C.1.a.(2) & (3), Column D or E by USDA classification):

I am conducting biomedical experiments which may potentially cause more than momentary or slight pain or distress to animals that WILL or WILL NOT (circle one) be relieved with the use of anesthetics, analgesics and/or tranquilizers. I have considered alternatives to such procedures, however, using the methods and sources described in the protocol, I have determined that alternative procedures are not available to accomplish the objectives of this proposed experiment.

(Principal Investigator Signature)

H. Scientific Review: This proposed animal use protocol has received appropriate peer scientific review, and is consistent with good scientific research practice.

(Division Chief Signature)

X. **Enclosures:** Appendix 1 - Pain Assessment Guidelines

Appendix 1 - PAIN ASSESSMENT GUIDELINES

COMMON CLINICAL SIGNS INDICATING PAIN, DISTRESS, OR DISCOMFORT IN EXPERIMENTAL ANIMALS

SYSTEM	SIGNS
Cardiovascular	Heart rate altered: pulse quality affected: Peripheral circulation decreased, blue and cold extremities (ears, paws).
Respiratory	Abnormal breathing pattern, rate and depth altered, labored, panting, nasal discharge.
Digestive	Bodyweight loss or poor growth: feces altered in volume, color or consistency (e.g., black with blue: pale; lack of bile pigments, undigested food: diarrhea/ constipation): vomiting, jaundice, salivation.
Nervous and musculoskeletal (locomotory)	Twitching, fitting, tremors, convulsions, paralysis, pupils dilated, shivering, hyperaesthesia, reflexes sluggish or absent: unsteady gait, lameness, muscle flaccidity, rigidity or weakness, protecting affected area such as "boarding" abdomen or reluctant to move a limb (e.g., arthritis).
Miscellaneous	Any abnormal swelling protrusion (hernia, rupture) or abnormal discharges from natural orifices;raised body temperature. Dehydration; sunken eyes, skin tents. Urine specific gravity increase, decrease in volume.

From: Morton, D.B. and P.H.M. Griffiths (1985) Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Vet. Rec. 116: 431-436.